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⑯ **Transfusional emulsion.**

⑯ There is disclosed a transfusion emulsion comprising fat, an emulsifier and water, 10-50 w/v% of said fat component being  $\alpha$ -linolenic acid ester. The transfusion emulsion has a fat component of improved balance and is suitable for nutritional supplement. Moreover, the present preparation is also useful as an antithrombotic agent or a cholesterol-lowering agent. Preferable  $\alpha$ -linolenic acid ester is triglyceride or ethyl ester of  $\alpha$ -linolenic acid.

**EP 0 145 873 A2**

Title of the Invention

Transfusion emulsion

Background of the Invention

## 1. Field of the Invention

5 The present invention relates to an improved transfusion emulsion.

More particularly, it is concerned with a transfusion emulsion in which 10 - 50 w/v% of the fat component is an  $\alpha$ -linolenic acid ester.

10 The transfusion emulsion of the invention is administered to patients, usually by drip infusion into the vein, for nutritional supplement. Moreover, the transfusion emulsion of the invention is useful as an anti-thrombotic or cholesterol-lowering agent.

## 15 2. Description of the Prior Art

The transfusion emulsion is an emulsion preparation composed of fat, emulsifier and water. There is employed in the prior-art transfusion emulsions purified soybean oil or purified safflower oil as the fat component.

20 Fatty acids contained in such fat components are mainly linoleic acid, and  $\alpha$ -linolenic acid is contained therein in a proportion as low as approximately 7% at highest.

Linoleic acid is converted in the human body to

arachidonic acid which is known to cause thrombin in the lungs when administered to rabbits. On the other hand,  $\alpha$ -linolenic acid is converted in the human body to eicosapentaenoic acid which is known to possess anti-  
5 thrombotic activity. Therefore, the prior-art transfusion emulsions which contain linoleic acid in a larger proportion and  $\alpha$ -linolenic acid in a smaller proportion can not be a nutritionally balanced transfusion emulsion. They are unsuitable especially for use in patients who  
10 are liable to thrombosis.

Summary of the Invention

As a result of extensive studies, we have found that a transfusion emulsion superior as an intravenous nutritional supplement is obtained by incorporating  $\alpha$ -linolenic acid in a proportion of 10 - 50 w/v% of the fat component and that such transfusion emulsion possesses a cholesterol-lower activity as well as an anti-thrombotic activity. The present invention is one completed on the basis of the above findings.

20 It is an object of the present invention to provide a transfusion emulsion in which fat components are nutritionally balanced.

Another object of the invention is to provide a transfusion emulsion which possesses a cholesterol-

lowering activity.

Detailed Description of the Invention

The present invention relates to a transfusion emulsion which is composed of fat, emulsifier and water and 5 in which 10 - 50 w/v% of the fat component is an  $\alpha$ -linolenic acid ester.

As the  $\alpha$ -linolenic ester, triglyceride or ethyl ester of  $\alpha$ -linolenic acid is preferable. As the oil containing  $\alpha$ -linolenic acid in a higher proportion is 10 mentioned, for example, linseed oil. In the present invention, triglyceride of  $\alpha$ -linolenic acid synthesized from  $\alpha$ -linolenic acid and glycerin may be employed or linseed oil may be employed after isolating triglyceride of  $\alpha$ -linolenic acid or as it is without isolation. 15 Ethyl ester of  $\alpha$ -linolenic acid are obtained by reacting  $\alpha$ -linolenic acid with ethyl alcohol or by transesterification by reacting triglyceride of  $\alpha$ -linolenic acid with ethyl alcohol. As the fat component other than the  $\alpha$ -linolenic ester may be used those purified vegetable oils which have been used as the fat component 20 of transfusion emulsions such as purified soybean oil and purified safflower oil.

Any of the emulsifiers which have been used as the emulsifier in transfusion emulsions may be employed in 25 the invention.

In addition, an appropriate amount of an emulsion stabilizer or an emulsion promotor such as glycerin or oleic acid may be incorporated.

The transfusion emulsion of the invention is preferably composed of 5 - 20 w/v% of the fat component, 1 - 2 w/v% of the emulsifier and the balance of water, and 10 - 50 w/v%, preferably 10 - 30 w/v% of the fat is an  $\alpha$ -linolenic ester. The emulsion stabilizer or the emulsion promotor is incorporated usually in the range from 10 1 to 5 w/v%.

The transfusion emulsion of the present invention is prepared according to conventional procedures. Pre-determined amounts of the components are blended, and to the blend is added an alkali. The resulting mixture 15 is dispersed in a homomixer to a homogeneous dispersion, to which distilled water is added. The resulting mixture is emulsified in a high pressure jet emulsifier to give a transfusion emulsion. The transfusion emulsion is divided into plastic bags, which are steam sterilized 20 under high pressure and vacuum packed with film to give the final product.

It was confirmed that the transfusion emulsion of the invention which contained an  $\alpha$ -linolenic ester in a higher portion exerted a platelet aggregation-inhibiting 25 action when intravenously administered. Accordingly,

the transfusion emulsion of the invention is useful not only as a balanced liquid preparation for nutritional supplement but also as an antithrombotic preparation.

Although it is known that  $\alpha$ -linolenic esters per se

5 possess a platelet aggregation-inhibitory activity, no antithrombotic transfusion emulsion containing the same in a higher proportion has been disclosed.

Furthermore, the transfusion emulsion of the invention produces an action lowering blood cholesterol level.

10 The cholesterol-lowering action of  $\alpha$ -linolenic esters is a novel finding unknown in literatures. The transfusion emulsion is useful for treatment or prevention of arteriosclerosis caused by the cholesterol existing in excess in blood due to excess intake of cholesterol or

15 disturbances of cholesterol metabolism, namely, aortic or coronary sclerosis, myocardial infarct, cerebral thrombosis, aortic aneurysm, obstruction of peripheral arteries, hyperlipemia, diabetes and the like.

For use of the transfusion emulsion of the invention as nutritional supplement, it is administered in exactly the same way as with prior-art transfusion emulsions. For use as an antithrombotic agent or an cholesterol-lowering agent, the transfusion emulsion is administered usually at a dose of  $\alpha$ -linolenic ester

25 of 1 - 30 g., preferably 3 - 10 g., 200 - 500 ml. in

terms of the liquid volume per day in adults, if required, in 1 - 3 divided doses, the dosage being variable depending upon age, bodyweight and conditions of the patient.

5 The present invention will be described in more details below with reference to Examples and Test Examples.

Example-1.

A mixture of 50 g. of ethyl  $\alpha$ -linolenate, 450 g. of purified soybean oil, 60 g. of purified yolk lecithine and 50 mL. of 0.1N-sodium hydroxide is dispersed in a 10 homomixer, followed by addition of injectable distilled water to a total liquid volume of 5 L. The resulting mixture is emulsified in a high pressure jet emulsifier to prepare a transfusion emulsion. The transfusion emulsion is divided into plastic bags in 200-mL portions. 15 The bags are steam sterilized under high pressure at 118°C. for 22 min. to prepare a transfusion emulsion preparation. After sterilized, the bags are vacuum packed in OV-film (oriented vinylon-film, manufactured by Unitika).

20 Example-2.

A mixture of 100 g. of  $\alpha$ -linolenic acid triglyceride, 300 g. of purified soybean oil, 48 g. of purified yolk lecithine, 2.0 g. of oleic acid, 100 g. of concentrated

glycerin and 10 ml. of 0.1N-sodium hydroxide is dispersed in a homomixer, followed by addition of injectable distilled water to a total liquid volume of 4 l. Then, the same treatments as in Example-1 are applied to produce  
5 a transfusion emulsion preparation.

Example-3.

A mixture of 80 g. of ethyl  $\alpha$ -linolenate, 320 g. of purified safflower oil, 48 g. of purified yolk lecithine, 2.0 g. of oleic acid, 100 g. of concentrated glycerin  
10 and 40 ml. of 0.1N-sodium hydroxide is dispersed in a homomixer, followed by addition of injectable distilled water to a total liquid volume of 4 l. Then, the same treatment as in Example-1 are applied to produce a transfusion emulsion preparation.

15 Test Example 1.

Platelet aggregation-inhibitory activity

Twenty-five Sprague Dawley rats weighing about 200 g. were used. They were divided into five 5-rat groups. Rats of Group 1 were intravenously administered  
20 at the tail with 18 ml. of a commercial transfusion emulsion containing 10 w/v% of soybean oil daily for 8 days. In addition, the rats were freely fed with a solid powder feed (CE-2; manufactured by Nihon Clea

Co., Ltd.). Rats of groups 2 - 4 were daily administered with 18 ml. of the transfusion emulsion prepared by the method described in Examples-1 to 3 in the same way as above for 8 days. The solid powder feed was also given 5 ad lib. Rats of Group 5 were freely fed with the solid powder feed. All the animals were fasted for 12 hours after the final intravenous administration and, under 5% Nembutal anesthesia, were taken each in a volume of 4.5 ml. from the abdominal aorta by means of a 20 G 10 needle into a syring in which 0.5 ml. of heparin had been placed. A PRP (Platelet Rich Plasma) of 300,000 platelets/ $\mu$ l. was prepared for each rat in a conventional manner. In a cuvette was placed 225  $\mu$ l. of the PRP from each rat. To the cuvette, after warmed at 37°C. for 5 15 min., was added 25  $\mu$ l. of collagen, an aggregation inducer to a final concentration of 50  $\mu$ g./ml. and measurement was made for platelet aggregation by means of an aggregometer. The results are given in Table 1. As shown in Table 1, the average rates of aggregation 20 were 60.5%, 55.7% and 64.7% for the rats of Group 2, 3 and 4, respectively, receiving the transfusion emulsion prepared in Examples 1, 2 and 3, respectively. Those data represented a significant inhibition as compared with the rats of Group 1 receiving the commercial 25 transfusion emulsion containing soybean oil and the rats of Group 5 administered an ordinary powder feed only.

Table 1.

<u>Group</u>	<u>Transfusion</u>	<u>Percent</u>	<u>Average</u>
	<u>emulsion</u>	<u>platelet</u>	<u>percent</u>
		<u>aggregation</u>	<u>aggregation</u>
1	Commercial	1	75.1
	soybean oil	2	84.1
	transfusion	3	78.1
	emulsion	4	79.4
		5	67.6
2	Example 1	6	59.1
		7	61.3
		8	72.0
		9	57.8
		10	52.3
3	Example 2	11	53.7
		12	44.8
		13	60.3
		14	73.5
		15	46.4
4	Example 3	16	71.8
		17	59.1
		18	53.5
		19	64.0
		20	75.2
5	Solid	21	86.3
	powder	22	83.4
	feed	23	97.5
		24	82.5
		25	93.8

## Test Example 2.

Blood cholesterol-lowering action

Groups of 5 male rats (Sprague Dawley) 5 weeks old were used in the experiment. The rats were intravenous-

5      ly administered at the tail each with 15 ml. of the transfusion emulsion prepared in Example 1, 2 or 3 above over 1 hour for 4 days. In addition, from the day on which administration of the transfusion emulsion was initiated, 10 ml. of 2 w/v% cholesterol suspension 10      in 0.5 w/v% aqueous solution of metrose (Trade name; manufactured by Sin-etsu Chemical Industries Co., Ltd.) was orally administered twice a day for 4 days. After the 4-day administration, the animals was fasted. On the 5th day, blood was drawn from the abdominal aorta, 15      and serum was separated by centrifugal separation and assayed for serum cholesterol by an enzymatic method (Assay Kit: V-cholestase; manufactured by Nissui Pharmaceutical Co., Ltd.). Results are shown in Table 2.

Table 2.

<u>Transfusion emulsion</u>	<u>Serum cholesterol (mg./dl.)</u>
Example 1	213.7 ± 30.6
Example 2	198.6 ± 24.7
Example 3	221.3 ± 28.1
Comparative Example	316.5 ± 57.8

As clearly seen from Table 2, the antiarteriosclerotic preparation containing an  $\alpha$ -linolenic acid ester according to the invention exerts a superior blood cholesterol-lowering action to the preparation of Comparative Example.

5 All the preparations of Examples 1, 2 and 3 are significantly different from that of Comparative Example with 5% significance level by the t test.

Acute toxicity

Tests were made in male Sprague Dawley rats to find 10 that the antiarteriosclerotic preparation according to the invention is very safe being similar to commercial transfusion emulsion containing soybean oil. Results are shown in Table 3.

Table 3.

Animal (sex)	Route of adminis- tration	Rate of infusion (ml./kg./min.)	Lethal dose (ml./kg.)			
			Example 1	Example 2	Example 3	Comparative Example
Rat (male)	Tail vein	7.5	204	196	187	192

What is claimed is:

1. Transfusion emulsion comprising fat, an emulsifier and water, 10 - 50 w/v% of said fat component being an  $\alpha$ -linolenic acid ester and prepared being suitable for intravenous transfusion.
2. Transfusion emulsion for intravenous transfusion according to Claim 1 wherein said  $\alpha$ -linolenic acid ester is triglyceride or ethyl ester of  $\alpha$ -linolenic acid.
3. Transfusion emulsion for intravenous transfusion according to Claim 1 or 2 wherein said transfusion emulsion is a cholesterol-lowering agent.